http://dx.doi.org/10.22161/ijeab.4435 ISSN: 2456-1878

Methionine Modulated Bioavailability of Inorganic Zinc ($ZnSO_4$. $7H_2O$) in Common Carp (*Cyprinus carpio*L.) Through Diets Containing Tricalcium Phosphate

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Abstract– *The present work has been conducted to study the efficacy of methionine modulated bioavailability of dietary inorganic Zn ($ZnSO_4$. $7H_2O$) in the fingerlings of common carp (Cyprinus carpio L.). The experiment was performed in triplicate for which young ones of common carp of average 3.39±0.68g weight and 6.02±0.25 cm length were stocked in the indoor glass aquaria (60× 30 ×30cm) @11 fish/ aquarium. Five diets including one control (D_1) and four experimental diets (D_2 to D_5) were formulated. In treatment diets D_2 and D_3 $ZnSO_4.7H_2O$ was added @88.4 and 176.8mg/kg, while in D_4 and D_5 $ZnSO_4.7H_2O$ was added @88.4 and 176.8mg/kg along with 1% DL Methionine as additional, respectively. Crude protein content in the experimental diets ranged between 36.68 - 39.14%, while zinc concentrations in diets (D_1 to D_5) were recorded 31.80, 57.40, 61.60, 56.60 and 62.80 mg kg^{-1}, respectively. Highest growth w.r.t. net weight gain was recorded (4.01g) and SGR (0.92%), feed conversion ratio (2.42) and protein efficiency ratio (1.08) was also recorded highest in fish fed with diet D_5. Moisture content (%) in fish flesh ranged between 77.20 – 78.90, protein 14.80 – 16.70, lipid 1.75 – 2.73, ash 1.82 – 2.61 and carbohydrate 1.13 – 1.92% among the fish fed with diet D_1 to D_5. Zinc concentration was recorded significantly high in muscle (36.90 mg kg^{-1}), liver (60.40 mg kg^{-1}) and bone (109.56 mg kg^{-1}). The present study indicates that Zn uptake in different tissues has been significantly improved due to addition of methionine in the formulated diets in young ones of common carp.*
Keywords— Common carp, Cyprinus carpio, Methionine, Tricalcium phosphate, Zinc uptake.

I. INTRODUCTION

Minerals serve as an essential requirement of the variety of functions, as both intra and extra cellular components. Zinc is an essential as micro mineral which is directly or indirectly involved in a wide variety of physiological processes including growth, development, reproduction and immune function (Watanabe *et al*.,1997). In fish, its deficiency leads to poor growth, high mortality, erosion of fins and skin, low content in bone (Takeuchi *et al*., 2002). Common carp (*Cyprinus carpio L.*) is an important fresh water carp, cultured across the world. Success of fish culture depends on rearing of quality seed fed with nutritionally balanced diet. For quality seed production; its young ones must be fed with nutritionally balanced diets. Fish meal is always considered as one of the best protein sources containing all essential amino acids, hence are widely used in fish diets. Common carp requires high levels of dietary protein during early stage of life cycle, for this

http://dx.doi.org/10.22161/ijeab.4435 ISSN: 2456-1878

addition of supplementary protein of animal origin in the formulated diets is a common practice. Rendered (recycled) by-products from animal waste serve as cheap source of quality protein (El Seyed 1998), but their application is limited due to low digestibly and restricted bioavailability of nutrients, particularly minerals due to certain limiting factors (Cho *et al*.,1982; Gill, 2000). Diets containing animal protein especially fish meal contains Tricalciumphosphate (TCP), which acts as inhibitory factor for Zn uptake in fish(Davis *et al*.,1993).

Organic compounds or chelated forms are important source of trace minerals, because they protect trace elements from forming insoluble complexes (such as phytate and TCP) in the digestive tract and facilitate transport across the intestinal mucosa (Ashmead1993). During production process of fish meal, calcareous compound present in fish converts into calcium phosphates and their derivatives (Butler and Gross 2017), even though fish scales also form a variety of calcium phosphate salts (Belouafa et al 2017). Methionine is such sulfur containing amino acids essentially required in fish diets, especially those containing high levels of plant protein source (Mai *et al*.,2006). Being a wide cultivable fish species in India as well as in the world, common carp is still not well studied fish species with respect to response against the inhibitory effect of TCP, moreover to find out the possibilities to mitigate the negative effect of TCP through amino acid modulation.

Keeping in view the above facts, the present work had been conducted to study theefficacy of methionine modulated bioavailability of dietary inorganic Zn ($ZnSO_4 . 7H_2O$) in the fingerlings of common carp (*Cyprinus carpio L.*).

II. MATERIALS AND METHODS

The experiment was conducted at the Department of Aquaculture, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The experiment was conducted in triplicate through rearing the young ones of common carp of average 3.39±0.68g weight and 6.02±0.25 cm length in the indoor glass aquaria (60× 30 ×30cm) @11 fish/ aquarium. Five diets including one control (D_1) and four experimental diets (D_2 to D_5) were formulated and prepared by mixing different ingredients such as casein, dextrin, gelatin, fish meal, sodium alginate, soybean meal, oil, carboxy methyl cellulose, Zn free mineral, vitamin mix, zinc sulphate ($ZnSO_4 . 7H_2O$), TCP and DL-methionine as per ratio given in table 1. Fishes were fed with prepared diets in crushed crumble form two split doses @ 2% of fish body weight (BW) for 90 days. Physico-chemical parameter of water was observed for temperature, pH, dissolved oxygen, total alkalinity and total hardness as per standard methods given in APHA(2005).

Proximate composition of feed was carried out as per AOAC, 2000, while proximate composition of flesh was estimated for protein Lowery *et al*.(1951), lipid Folch*et al*.(1957), moisture and ash AOAC, (2000). Zinc concentration in flesh, liver and bone was analysed through atomic absorption spectrophotometer (Elico) as per standard method as described by Jorhem and Engman,(2000).

Growth in terms of net weight and length gain was calculated on the basis of difference in values at the start and end of experiment. Specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) was calculated as per formula given below

$$\text{SGR (\% increase in weight /day)} = \frac{\ln \text{Final body wt. (g)} - \ln \text{Initial body wt. (g)}}{\text{No. of days of experiment}} \times 100$$

$$\text{FCR} = \frac{\text{Feed given (g)}}{\text{Weight gain (g)}}$$

$$\text{PER} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

STATISTICAL ANALYSIS

Data recorded for Physico-chemical parameters of water, Body weight and growth parameters of fish, biochemical composition of flesh and change in zinc concentration (mg kg^{-1}) in different tissues were analysed by two-tailed bivariate Pearson's correlation coefficient for average values, standard deviation and correlation coefficient using one way ANOVA by SPSS 16.00 software.

III. III. RESULTS AND DISCUSSION

Fish, being an aquatic vertebrate is directly influenced by water quality parameters for their survival and growth. During the experimental period, amongphysico-chemical

http://dx.doi.org/10.22161/ijeab.4435 *ISSN: 2456-1878*

parameters temperature, pH and dissolved oxygen (DO) varied within the desirable range for fish culture as suggested by Boyd(1990) and Bhatnagar and Devi (2013) for warm water fish species. However, total alkalinity and hardness were slightly higher than optimum range but no negative effect in terms of behavior recorded in fish. Details of the water quality recorded during the study period are given in table 2.

Crude protein content in the fish fed with different experimental diets ranged between 36.68-39.14%, while zinc concentration in diets (D_1 to D_5) ranged 31.80, 57.40, 61.60, 56.60 and 62.80 mg kg^{-1}, respectively (Table 3).In diets, highest crude protein content recorded in D_5, followed by D_4, it may be possibly due to addition of methionine; which is itself a nitrogen containing biomolecule i.e. amino acid. Higher concentration of zinc in prepared diets as compared to rate of addition may be due to Zn content naturally present in different feed ingredients in different ratio. Garling and Wilson (1976) suggested that 25 – 36% crude protein as optimum level in diets for the warm water fishes, while Jader and Al-Sulevany (2012) reported the highest growth in common carp juveniles, when fed with 30% crude protein. Paul and Giri (2015) advocated the protein requirement in-between 25-35% for the optimum growth of fish. Singh *et al*. (2018) reported the highest growth in youngones of common carp @ 35% Crude protein level. In present study, protein content in experimental diets was within the range of protein requirement of common carp suggested by different workers.

The growth in fish stocked during experimental period was assessed in terms of net weight gain (NWG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) of fish for each treatment were calculated (Table 4).

Highest weight gain recorded in fish fed with D_5 diet; however in D_3diet; reduced growth was recorded which may be possibly due to negative effect of tricalcium phosphate, which was added but no compound was added to cater the negative effect of TCP, however in treatment D_2 and D_5 effect was comparatively less which may be either due to either less concentration of TCP of addition of methionine. Similarly SGR, FCR and PER values also improved in fish fed with D_5 diet, it may be due to addition of methionine. The values recorded in present study were well close to the observations recorded by different workers. Sultana *et al*.(2001) reported the SGR in between 2.53 – 3.24, FCR 1.22 – 1.78 and PER 1.68 – 2.48 in common carp fry fed with 33.34% crude protein @ 5% body weight (BW) for 45 days. Kiaalvan di*et al*.(2011) reported the FCR in between 4.76 – 6.25 and PER 0.38 – 0.47 in common carp juveniles (8.6 g) fed with 26 – 28% crude protein @ 5% BW daily while Jader and Sulevany (2012) recorded the SGR 0.71 – 0.87, FCR 2.27 – 3.01 and PER 0.79 – 1.05 in juveniles of common carp when fed with 25-35% crude protein.

The Caracas composition to evaluate flesh for moisture, protein, lipid, ash and nitrogen free extract (NFE) recorded at the time of stocking and termination of experiment. With the progress of experiment; significant improvement in protein, lipid, ash and NFE content recorded (Table 5). After termination of experiment, moisture content (%) in fish flesh ranged between 77.20 – 78.90, protein 14.80 – 16.70, lipid 1.75 – 2.73, ash 1.82 – 2.61 and carbohydrate 1.13 – 1.92% among D_1 to D_5, however values for above mentioned parameters were significantly less ($P \leq 0.05$), except moisture content.

Zinc concentration in flesh, liver and bone analyzed at the start of experiment and after termination of experiment. Zinc concentration in fish flesh ranged in between20.10 – 36.90, in liver 20.10 – 62.20 and in bone 89.70 – 109.56 $mgkg^{-1}$ at the time of termination in diets D_1 -D_5, however at the time of stocking it was 18.60, 17.40 and 84.70 mg kg^{-1} in flesh, liver and bone, respectively (Table 6). Significantly high ($P \leq 0.05$) concentration of zinc in fish flesh fed with D_6 diet recorded, it may due to added Zn supplement at higher levels. In addition to this, it may also possible that absorption of zinc might be absorbed through gut in the presence of methionine. In liver, highest levels of Zn concentration recorded in fish fed with diet D_4.

IV. CONCLUSION

In the present study, significantly high ($P \leq 0.05$) NWG, SGR, FCR and PER in diet D_5 indicates the mitigation of inhibitory effect compound present in diet. Tricalcium phosphate (TCP) is a proven inhibitory factor, which reduces the Zn uptake in tissue. The present study indicates that Zn uptake can be improved through addition of methionine even though at 1% level in the formulated diets for young ones of common carp.

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http://dx.doi.org/10.22161/ijeab.4435 ISSN: 2456-1878

Table. 1: Composition of experimental diets (%)

Ingredients	**Diets**				
	D1 Control (0mg Zn kg^{-1})	**D2 (20mg Zn kg^{-1})**	**D3 (40mgZn kg^{-1})**	**D4 (20mg Zn kg^{-1})**	**D5 (40mg Zn kg^{-1})**
	D_1	**D_2**	**D_3**	**D_4**	**D_5**
Casein	20	20	20	20	20
Dextrin	30	30	30	30	30
Gelatin	8	8	8	8	8
Fish Meal	10	10	10	10	10
Sodium Alginate	12	12	12	12	12
Soybean Meal	8	8	8	8	8
SoybeanOil	2	2	2	2	2
Carboxy Methyl Cellulose	5	5	5	5	5
Zinc Free Mineral*	3	3	3	3	3
Vitamin Mix**	2	2	2	2	2
$ZnSO_4.7H_2O$ (mg/kg diet)	0	88.4	176.8	88.4	176.8
Tri Calcium Phosphate***	0	2	2	2	2
DL Methionine***	0	0	0	1	1

*$FeSO_4.H_2O$: 41.16, $CuSO_4$:0.51, $CaCO_3$: 20.58, KIO_3: 10.56, $MgSO_4$: 44.50, $MnSO_4.H_2O$: 2.29, NaCl: 17.15(g kg^{-1} diet).

**Vitamin B2: 1.25g, Vitamin B6: 0.5g, Vitamin B12: 6.25 mg, Biotin: 12.5mg, Cal. Pantothenate: 1.25g, Niacinamide: 37.5g, Base: q.s. (g $100g^{-1}$)

***Added as over and above 100%

http://dx.doi.org/10.22161/ijeab.4435 ISSN: 2456-1878

Table. 2:Physico-chemical parameters of water

Parameters	Range
Temperature (°C)	29.1 – 29.6
pH	8.41 – 8.48
Dissolved oxygen (mg l^{-1})	7.96 – 8.64
Total alkalinity (mg l^{-1})	260.00 – 271.00
Total Hardness (mg l^{-1})	275.00 – 287.00

Table. 3: Proximate composition (%) of formulated diets (on DM basis)

Component	Diets				
	D_1	D_2	D_3	D_4	D_5
Moisture	8.88	8.98	9.12	8.66	8.54
Crude protein	36.68	37.12	36.28	38.66	39.14
Crude fat	2.12	1.98	2.22	2.44	2.56
Ash	9.14	9.28	11.00	10.62	11.24
Crude fiber	3.41	3.43	3.42	3.52	3.50
NFE	39.76	39.20	37.95	36.10	35.01
Zn (mg kg^{-1})	31.80	57.40	61.60	56.60	62.80

*Variation in proximate composition may be due to addition of TCP in D2, D3 and TCP with methionine in D4 and D5.

Table.4: Body weight (BW) and growth parameters of fish in different treatments during the experimental period (Mean ±SE)

Month	Diets				
	D_1	D_2	D_3	D_4	D_5
NWG	2.85^a±0.69	2.96^a±0.50	2.19^a±0.24	3.35^a±0.89	4.01^a±0.42
SGR	0.56^a±0.12	0.72^a±0.21	0.48^a±0.65	0.69^a±0.20	0.92^a±0.13
FCR	4.54^a±0.90	5.68^a±2.86	5.68^a±1.78	3.71^a±1.05	2.42^a±0.30
PER	0.64^a±0.13	0.71^a±0.25	0.56^a±0.13	0.81^a±0.23	1.08^a±0.12

* Values with different alphabetical superscripts differ significantly within row ($P \leq 0.05$)

** Values with different numerical superscripts differ significantly within column ($P \leq 0.05$)

http://dx.doi.org/10.22161/ijeab.4435 ISSN: 2456-1878

Table.5: Change in biochemical composition (%) of common carp flesh (Mean± SE)

Parameters (%)	Initial	D_1	D_2	D_3	D_4	D_5
Moisture	$82.10^{a}\pm0.27$	$78.90^{b}\pm0.24$	$78.10^{cd}\pm0.35$	$78.00^{cd}\pm0.49$	$77.60^{de}\pm0.14$	$77.20^{e}\pm0.02$
Total proteins	$13.60^{d}\pm0.08$	$14.80^{c}\pm0.06$	$15.60^{b}\pm0.00$	$15.60^{b}\pm0.03$	$16.60^{a}\pm0.06$	$16.70^{a}\pm0.15$
Total lipids	$1.97^{cd}\pm0.21$	$2.73^{a}\pm0.06$	$2.27^{bc}\pm0.08$	$2.00^{cd}\pm0.00$	$2.08^{c}\pm0.03$	$1.75^{d}\pm0.05$
Ash	$1.40^{e}\pm0.05$	$1.82^{d}\pm0.07$	$2.52^{ab}\pm0.01$	$2.32^{c}\pm0.01$	$2.48^{b}\pm0.00$	$2.61^{a}\pm0.01$
Total carbohydrates	$0.77^{de}\pm0.27$	$1.67^{abc}\pm0.24$	$1.41^{abc}\pm0.17$	$1.92^{a}\pm0.07$	$1.13^{cd}\pm0.21$	$1.70^{ab}\pm0.31$

* Values with different alphabetical superscripts differ significantly within row ($P \leq 0.05$)

Table. 6: Change in zinc concentration (mg kg^{-1}) in different tissues of common carp (Mean± SE)

Fish organ	Initial	D_1	D_2	D_3	D_4	D_5
Flesh	$18.60^{f}\pm0.14$	$21.20^{d}\pm0.01$	$20.10^{e}\pm0.02$	$25.40^{c}\pm0.02$	$35.70^{b}\pm0.02$	$36.90^{a}\pm0.03$
Liver	$17.40^{f}\pm0.01$	$20.10^{e}\pm0.01$	$35.60^{d}\pm0.01$	$36.40^{c}\pm0.01$	$62.20^{a}\pm0.00$	$60.40^{b}\pm0.01$
Bone	$84.70^{f}\pm0.005$	$89.70^{e}\pm0.05$	$96.42^{c}\pm0.01$	$94.92^{d}\pm0.02$	$106.70^{b}\pm0.01$	$109.56^{a}\pm0.01$

* Values with different alphabetical superscripts differ significantly within row ($P \leq 0.05$)